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THE *Ac/Ds* TRANSPOSON SYSTEM FROM MAIZE AS A TOOL FOR GENERATING MUTANT PHENOTYPES IN TOMATO (*LYCOPERSICON ESCULENTUM*)

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ABSTRACT

A non-targeted transposon tagging experiment was performed in tomato (Lycopersicon esculentum). The Ac/Ds transposon system from maize was used. To generate a transposon tagging population, tomato genotypes ET 570 and SLJ10512 were used. The ET 570 genotype contains a single homozygous T-DNA with Ds at the end of the long arm of chromosome 3. The SLJ10512 genotype carries a stabilized Ac that can activate Ds in trans. This tagging population was tested at the seedling level (Van der Biezen et al., 1996) and two phenotypic mutants were found that co-segregated with a Ds insertion: feebly (fb) and yellow jim (yj). In this work, performed under field conditions, all stages of individual plant development were screened for mutant phenotypes. Out of 269 families, with in total approximately 400 unique transposed Ds elements, 5 different phenotypes were observed. One of the phenotypes, fruit distortion, segregated in a Mendelian fashion. Plants with the fruit distortion phenotype did not produce seeds. In the case of the other 4 phenotypes, green spots, dark green leaves, dwarf and partially hairless plant only one phenotypic mutant plant per family was obtained and, thus, could not be classified. Phenotypic mutants, obtained from this transposon tagging experiment, that show linkage to the Ds insertion, might be used to isolate the genes responsible for the mutant phenotypes.

Introduction

The cultivated tomato *(Lycopersicon esculentum)* is widely used as a model plant in genetic studies. The tomato is an important vegetable crop and is genetically well characterised (14, 15). For genetic studies it is important to have a wide range of induced mutants. X-rays and ionising radiation can cause mutations in all organisms (6). Ethyl methansulphonate (EMS) is an alkylating agent and has been used effectively to obtain mutants in tomato (19). Another tool to obtain phenotypes is genetic variation among plants regenerated from cells and tissues. This so-called somaclonal variation (17) can be due to gross alterations in chromosome number and structure, point mutations, mitotic recombinations, amplification, deletion, transposition or methylation of DNA sequences (13, 17). Recessive and some dominant monogenic mutants can be obtained as a result of somaclonal variation in tomato. The mutations can affect all aspects of plant life as fruit colour, leaf shape and phytochrome content (17).

In the last 10 years insertional mutagenesis took its place as a valuable research tool in a variety of systems including prokaryots and also eukaryots like animals and plants (1, 2).

***Abbreviations:** Ac/Ds - Activator/Dissociation; sAc - stable Activator; fb - feebly; yj - yellow jim; div - divaricata; dif determinate infertile; wt - wild type; hig - hygromycin; kan - kanamycin; PPT - phosphinothricin; BAR - phosphinothricin acetyltransferase gene; HPT - hygromycin phosphotransferase gene; GUS - b-glucoronidase gene; EMS - ethyl methansulphonate; cM - centiMorgans.

Insertional mutagenesis is a technique by which mobile or introduced DNA, with a known sequence, inserts into a gene. The inserted sequence not only creates a mutation but also genetically marks the affected gene, facilitating its isolation. Transposons, short mobile genetic elements, were the first type of insertional mutagens used for this purpose (5). Transposons are found in a wide variety of species and recently some plant transposons have been introduced into new plant hosts, expanding their use for genetic analysis (5, 8). The *Ac/Ds (Activator/ Dissociation)* system from maize has been successfully used for gene isolation in a number of heterologous hosts, including petunia (3), *Arabidopsis* (12), tobacco (22) and tomato (9).

The efficiency of a transposon tagging experiment depends on the transposon induced mutation frequency (20). Experiments for obtaining transposon induced mutants can either be targeted with the goal to recover a specific gene, or non-targeted, not aiming at a specific gene but for any phenotype that can correspond with the transposon insertion (21). Taking various parameters into account (20), the *Ds* induced visual mutation frequency in tomato can be estimated at 1×10^{-2} per transposition event.

Using the *Ac/Ds* system from maize in a non-targeted transposon tagging experiment in tomato and testing 420 families, Van der Biezen *et al.* (21) found 2 phenotypic mutants at the seedling level, co-segregating with a *Ds* insertion: *feebly (fb)* and *yellow jim (yj)*. To further analyse this tagging population in later stages of plant development, a field experiment was carried out to reveal flowering, fertility, fruit or other mutants that would appear later in the individual plant development.

Materials and Methods

1. Plant material.

Two tomato *(Lycopersicon esculentum)* genotypes ET 570 (91570) (11) and SLJ10512 (16), which carried single homozygous T-DNAs with *Dissociation (Ds)* and stable *Activator (sAc)*, respectively, were used to generate the tagging population (21). The *Ds* element has been placed between the Cauliflower Mosaic Virus 35S promoter and the phosphinothricin acetyltranspherase gene (BAR). In this way resistance to phosphinothricin (PPT) was used to follow the transposon excision (4). To monitor the *Ds* reintegration the internal 1.6 kb Hind III fragment of *Ac* has been replaced with a 2 kb P_{-nos} hygromycin phosphotransferase (HPT) gene fusion (10).

The Ac element contains a β-glucuronidase uidA (GUS) reporter gene (16). Both constructs are shown in **Fig. 1a.**

The *Ds* element has been mapped at the end of the long arm of chromosome 3 (18) (**Fig. 1c**) and the *sAc* element has been localised on chromosome 4 (16).

2. Crossing scheme and selection procedure

The parental lines SLJ10512 (*sAc*) and ET 570 (*Ds*) have been crossed to produce double hemizygous *sAc/Ds* F_1 plants (**Fig. 2b**). Two hundred and sixty four F_1 plants have been self-pollinated to transmit germinally transposed *Ds* elements in the F_2. About 25 000 F_2 plants have been analysed for phosphinothricin (PPT) resistance, hygromycin (Hyg) resistance and the absence of GUS activity corresponding respectively to *Ds*-excision, *Ds*-reinsertion and absence of *sAc* . Following this selection procedure, 420 F_2 plants were obtained, self-pollinated, and the F_3 progeny was screened for segregating phenotypes at the seedling level (21). In the field experiment, the progeny of 269 F_2 plants was used for screening segregating phenotypes.

3. Field experiment.

For the transposon tagging experiment screened under field conditions, the progeny of 269 F_2 tomato plants was used (**Fig. 1b**). Fourty seeds from each of the 269 F_2 plants were germinated to produce the F_3 tagging population. Twenty F_3 plants originating from one F_2 plant were transferred to the field 35 days after germination - 5380 plants in total on 1500 m^2. The progeny of every F_2

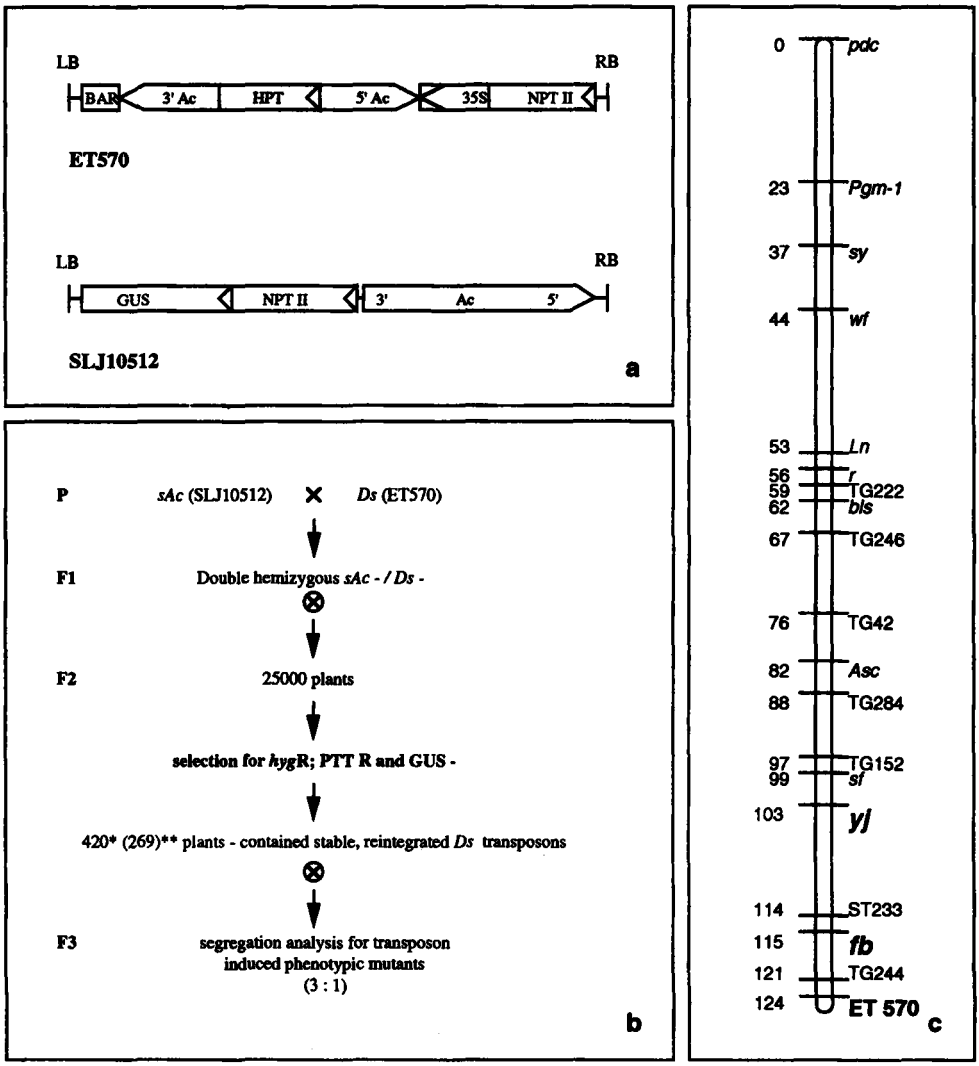

Fig. 1. a. T-DNA constructs. ET 570 (91570) - T-DNA which carried the *Ds* element. *Ds* is placed between the Cauliflower Mosaic Virus 35S promoter (35S) and the phosphinothricin acetyltranspherase gene (BAR). The internal 1.6 kb Hind III fragment of *Ac* has been replaced with the 2 kb P_{-nos} hygromycin phosphotransferase gene (HPT). SLJ10512 - T-DNA carried stable (deletion in 3' end) *Activator (sAc)* element. The T-DNA contains a β-glucuronidase uidA (GUS) reporter gene and a Kan^R gene. **b.** Crossing scheme and selection procedure. The parental genotypes SLJ10512 (*sAc*) and ET 570 (*Ds*) have been crossed to produce double hemizygous *sAc/Ds* F_1 plants. F_1 plants have been self-pollinated in order to transmit germinally transposed *Ds* elements to F_2 plants. F_2 plants have been selected for *Ds*-excision, *Ds*-reinsertion using selectable markers conferring resistance to phosphinothricin (PPT) and hygromycin (Hyg), respectively. Absence of GUS activity was used to select plants without transposase. As a result 420 F_2 plants have been selected and self-pollinated in order to produced F_3 families. F_3 families were screened for phenotypes segregating in a Mendelian fashion. *number of F_2 plants used in the seedling transposon tagging experiment; **number of F_2 plants used in the field experiment. **c.** Integrated map of tomato chromosome 3 (after Van der Biezen *et al.* 1994). The positions of the markers are expressed in centiMorgans (cM). Positions of *feebly (fb)* and *yellow jim (yj)* are according to the data from Van der Biezen *et al.* (1996) (22). The ET 570 - T-DNA construct with the *Ds* element is located at the end of the long arm of chromosome 3.

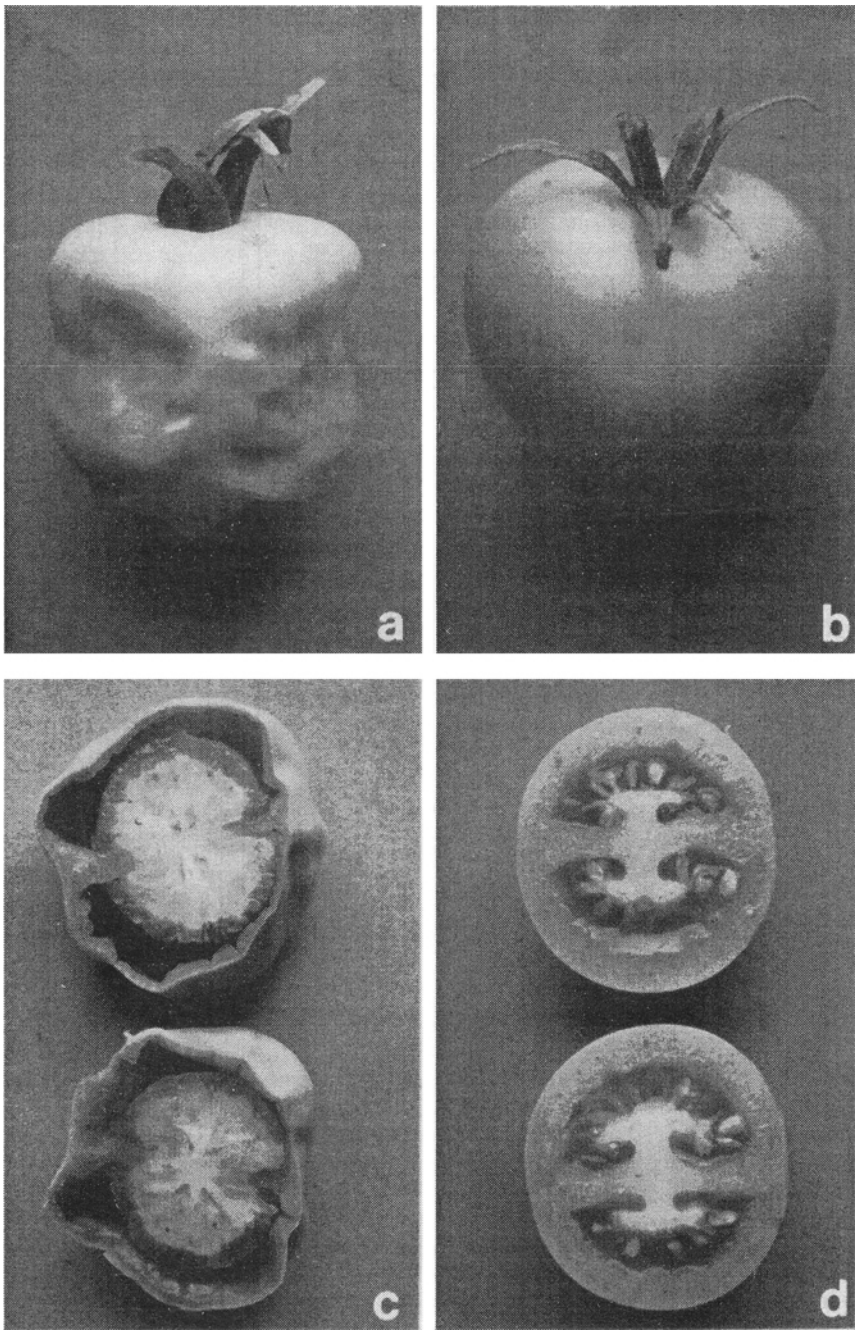

Fig. 2. *Fruit distortion* phenotype: **a.** *fruit distortion* tomato; **b.** wild type tomato; **c.** *fruit distortion* tomato - cross section; **d.** wild type tomato- cross section.

plant is further referred to as a family. The field experiment was carried out during a 4.5 month period, from May to September, 1996.

Results and Discussion

Using the *Ac/Ds* transposon system from maize a non-targeted transposon tagging experiment was performed in tomato. The experiment was carried out to reveal phenotypic mutants appearing in all stages of individual plant development. The plants were grown under field conditions. All together, 269 families were screened, about 20 plants per family. Within each family we looked only for visible alterations in the phenotype, segregating in a Mendelian fashion. Both recessive and dominant phenotypes due to *Ds* mutated alleles were expected. In the field experiment, we found 5 different phenotypes (**Table**). One of the phenotypes, *fruit distortion*, showed a recessive behaviour, segregating in a Mendelian fashion of 3 : 1. The remaining 4 phenotypes, *green spots* on unripe fruit skin, *dark green leaves*, *dwarf* and *partially hairless plant*, showed only one variant plant per family (Table). Phenotypes showing a dominant phenotypic effect were not observed.

TABLE

Mutant phenotypes appeared in the field experiment and their segregation pattern. χ^2 - chi-squire values for a 3 : 1 (wt : mutant phenotype) expectation ($p<0.05$ if $\chi^2>3.84$)

mutant phenotype	F_2 (family) number	segregation (wt : mutant phenotype)	χ^2
fruit distortion	26	17 : 3	1.06
	71	16 : 3	0.85
	73	17 : 3	1.06
	94	17 : 3	1.06
	111	18 : 2	2.40
	116	16 : 3	0.85
	161	15 : 3	0.66
	163	17 : 2	2.12
	172	17 : 3	1.06
	173	16 : 2	1.85
	191	15 : 4	0.15
	208	16 : 3	0.85
	221	16 : 3	0.85
fruit distortion	3, 19, 82, 186, 292	19 : 1	4.26
dwarf	52, 74, 75, 178, 313	19 : 1	4.26
partially hairless plant	37, 107, 166, 217	19 : 1	4.26
green spots	312	11 : 1	–
dark green leaves	311	7 : 1	–

Fruit distortion

In 18 families, the *fruit distortion* phenotype was observed (**Fig. 2**). In 13 families this *fruit distortion* phenotype segregated in a Mendelian fashion (Table).

Instead of normal seed production, the *fruit distortion* phenotype showed very small seed like structures. The fruits had empty locules (**Fig. 2c**). Some of the mutant fruits showed a different shape and an irregular skin (**Fig. 2a**). Fruits from the mutant plants did not have seeds and gel. Most often, fruits originating from the first inflorescence showed the irregular skin. In the same inflorescence it was possible to see fruits with both abnormal skin and shape and fruits only with angular shape. The fruits from the second and later inflorescences showed in most cases both wild type (wt) shape and skin, yet with empty locules and no seeds. The vegetative parts of the mutant plants were the same as in the wild type.

Other phenotypes observed

In 5 families (Table), *dwarf* plants were found. Small plants, no more than 60 cm in height, with shortened internodes and curly, dark green coloured leaves. These plants produced smaller fruits and these were retarded in ripening. The skin of the mature fruits showed irregular yellow spots and stripes. Seed production was normal. In all 5 families, only one dwarf plant out of 20 was found.

In the same field experiment, 4 plants out of 4 different families appeared to be *partially hairless* (Table). After the plant development started normally, two months later new parts of the plants appeared without hairs. Fruits from the hairless parts of the plants did not have seeds. In shape and co-

lour these fruits were wild type.

At the fruit stage of plant development, in family 312, a plant was found showing more dark green stripes and spots on the skin of the green fruits. During fruit ripening this pattern disappeared. In the same family, 7 plants showed an intermediate phenotype - very narrow stripes and small spots on the fruit skin. Due to the poor germination in this family, only 11 plants could be analysed and the segregation can, thus, not be considered reliable.

In family 311, two months after germination, a vigorous plant was found with darker green leaves. Mature fruits showed irregular yellow spots. Seed production of these plants was normal. Flowers did not show any abnormality.

Five different phenotypes were found in the experiment performed on the field. Only one of the phenotypes, *fruit distortion*, showed the expected segregation pattern of 3 : 1 (Table). The other 4 phenotypes, *green spots* on unripe fruit skin, *dark green leaves*, *dwarf* and *partially hairless plant* (Table), showed only one mutant per family. It was hard to draw a conclusion about the segregation patterns of the *green spots* and *dark green leaves* phenotypes because of their poor germination under field conditions. Therefore, *fruit distortion* was the promising phenotype for further molecular genetic analysis. Regarding the other four phenotypes found in the field experiment, further analysis of the families and their progenies is required to clarify the segregations.

In the genotype ET 570, *Ds* has been localised on the long arm of chromosome 3 (11) (Fig. 1c). Estimations on the basis of existing data indicate that 50 % of the *Ac/Ds* transpositions occur intrachromosomally within 10 cM of the original position (20). The long arm of chromosome 3 has a low abundance of known genes for which mutants are available (18), therefore the chances of "tagging" a known mutant phenotype are small. On the other hand, this experiment made it possible to scan this chromosome 3 region for putative genes resulting in new phenotypes. Screening a transposon-tagging population at the seedling level, Van der Biezen *et al.* (21) found two phenotypic mutants on chromosome 3 - *feebly (fb)* and *yellow jim (yj)*, both linked to the original transposon position. One of the 5 phenotypes found in the field experiment, *fruit distortion*, resembled *fb* at the fruit level. The other 4 phenotypes differed from any known mutants correlated with chromosome 3 (15). This can be expected since in a non-targeted transposon tagging experiment as described here there is a 50 % probability (20) for the *Ds* to integrate unlinked to reveal a new phenotype.

The *fruit distortion* phenotype resembled the *feebly (fb)* phenotype at the fruit stage. *Feebly* is another allele of the previously described mutant *divaricata* (14). *Feebly* shows a more severe phenotype at every stage of the plant development than *divaricata*. The *fb* phenotype shows weak growth. Mutant plants are characterised by crinkled leaves, poor fruit production and low seedset. Some of the fruits have irregular skins and unusual angular forms. Characterisation of the *fb* phenotype includes dark purple coloured lower sides of the first leaves caused by a 10-fold higher anthocyanin level. This excess of anthocyanin disappears later in plant development (21). Weak growth, crinkled leaves and higher anthocyanin level at the seedling stage were not observed in the case of *fruit distortion*. The *fruit distortion* resemblance to *div* and *fb* could be explained by a transposon insertion in the same *div* gene but at a different position than the *Ds* in *feebly*. Another possibility is a *Ds* insertion in another gene resembling *divaricata* in phenotypic expression.

The phenotype *fruit distortion* resembles the physiological disorder *puffiness*. *Puffiness* is the presence of open cavities between the outer walls and the locular contents in one or more locules of the tomato fruit. The severity of this physiological disorder depends on the genotype and growing conditions. Extreme conditions especially affect

greenhouse tomatoes (7). In this experiment a greenhouse crop, with a Moneymaker genetic background, was used to perform in the field where the environmental conditions differed considerably from the greenhouse and, therefore, plants were exposed to high stress. However, the clear Mendelian segregation pattern within most of the families showing *fruit distortion* (Table), indicated this disorder was caused by genetic differences. Further segregation analysis and analysis at the molecular level of the plants showing this phenotype will reveal the relationship between the mutant phenotype and a *Ds* insertion.

The observation that *fruit distortion* tomatoes gave very small seeds with embryo like structures (data not shown) indicated that fertilisation occurred and later on the seed developing process stopped.

This field experiment was carried out to reveal mutants that appeared later in individual plant development. One of the phenotypes, *dark green leaves*, concerned leaf colour and appeared two months after germination. *Partially hairless plant, fruit distortion* and *dwarf* were phenotypes concerning plant morphology and development - retarded in fruit ripening or no seed production. One of the phenotypes, *green spots*, showed differences in fruit colour during the ripening process. Compared to tomato phenotypes resulting from different mutagenic treatments (14, 17, 19), we did not obtain new classes of phenotypes. However, *Ds* could translocate to any part of the tomato genome, thereby revealing a new phenotype because of altered gene function. *Partially hairless plant* did not resemble any of the known phenotypes (14).

Three of the phenotypes appeared in more than one family of the tagging population (Table). One possible explanation was that repeating mutant phenotypes were a result of the same *Ds* insertion that happened early in one F_1 plant. The progeny of the first 24 F_1 plants was germinated independently but the progeny of the other 240 F_1 plants was pooled (21). Therefore, the appearance of the same phenotype more than once in the tagging population might be due to a *Ds* insertion, happened early in plant development of the same F_1 plant. However, one of the phenotypes, *fruit distortion,* was found in families No 3 and 19 originating from different F_1 plants and in 16 other families originating from pooled F_1 progeny, which indicated that at least 3 independent *Ds* insertions in the same gene were possible. This has also been observed in *Arabidopsis*. Using *Ac* in *Arabidopsis* Bhatt *et al.* (2) found that 6 out of 12 analysed *Dif* mutants were due to independent insertions into the DIF1 gene.

In two screens, the first performed previously at the seedling level in the green house (21) and the second performed in the field, 3 phenotypes *(feebly, yellow jim* and *fruit distortion)* segregating in a Mendelian fashion were revealed. The mutation frequency of around 1 % was mathematically predicted (20). In these experiments mutant phenotypes were screened visually and therefore biochemical and disease-resistance mutants, for instance, were missed. Other methods to obtain stable phenotypes in tomato such as treatment with chemical agents /EMS/ (19), different types of radiation (14) and somaclonal variation (17) can lead to higher mutation frequencies, but only insertional mutagenesis offers the opportunity to isolate the genes responsible for the mutant phenotypes.

Transposons as mutagens cause altering of gene function that might result in visible mutant phenotype. In spite of the great number of plants that had to be scored, the obtained mutant phenotypes serve as material for further genetic studies. Around 400 unique *Ds* transpositions resulted in one promising mutant phenotype obtained from the field experiment. The *fruit distortion* phenotype showed the expected segregation pattern. Further genetic and molecular analysis is required to prove that a specific *Ds* insertion is linked to the mutant phenotype.

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